Serotonin Receptor Subtypes: Biochemical, Physiological, Behavioral, and Clinical Implications

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Analysis of 5-HT₁ Binding Site Subtypes and Potential Functional Correlates

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Serotonin (5-hydroxytryptamine; 5-HT) has been implicated in a number of human diseases such as anxiety, depression, migraine, and epilepsy. However, a specific role for 5-HT has not been documented in any human disease. Receptor-site analysis is one approach which may elucidate the functional role of 5-HT in the central nervous system (CNS). Since the analysis of M and D receptors in 1957, it has become clear that multiple 5-HT receptors exist. In the past 3 years, a number of novel pharmacologic agents have been developed which have greatly facilitated the analysis of 5-HT receptor subtypes in the CNS. For example, 8-hydroxy-2-N,N-(di-propylamino)tetralin (8-OH-DPAT), RU 24969, ketanserin, and ritanserin have been shown to be potent and selective binding-site subtype agents.

At the present time, at least four distinct subtypes of 5-HT receptors have been identified in brain membranes (Hoyer et al., 1985b). The present report will focus on the current classification of 5-HT₁ binding-site subtypes. All 5-HT₁ binding-site subtypes can be labeled by ³H-5-HT. Radioligand binding studies have clearly demonstrated that total 5-HT₁ binding as defined by ³H-5-HT is heterogeneous in the rat frontal cortex (Pedigo et al., 1981). In studies performed in this laboratory (Peroutka, in press), computer analysis of drug competition curves with total ³H-5-HT binding shows that 5-HT competition with ³H-5-HT binding to total 5-HT₁ sites is consistent with a homogeneous receptor population. However, the interactions of d-LSD, (m-trifluoro-methylphenyl) piperazine HCl (TFMPP), methysergide, spiperone, and mianserin with total 5-HT₁ sites are more consistent with a two-site model of 5-HT₁ binding. In addition, computer-assisted curve fitting analysis of RU 24969 competition studies is most consistent with a three-site...
model of 5-HT₁ binding in rat frontal cortex. 8-OH-DPAT is approximately 10,000-fold selective for the 5-HT₁A site versus all other known 5-HT₁ binding site subtypes. As a result, 100 nM 8-OH-DPAT can be used to effectively block ¹H-5-HT binding to the 5-HT₁A site without markedly affecting the binding or ¹H-5-HT to non-5-HT₁A sites. Indeed, Scatchard analysis of ³H-5-HT binding in the absence or presence of 100 nM 8-OH-DPAT demonstrates a significant decrease in the Bₘₙ without markedly affecting the Kᵦ of ³H-5-HT in the rat frontal cortex. Analysis of residual ³H-5-HT binding reveals that the competition curve for RU 24969 is even more shallow than at total 5-HT₁ sites. Computer-assisted analysis reveals that the interactions of methysergide, TFMPP, mianserin, and RU 24969 are still consistent with at least a two-site model of non-5-HT₁A binding. No pharmacologic agent could be identified which has more than a 440-fold selectivity for subtypes of putative non-5-HT₁A binding.

As a result, 5-HT¹B binding in this laboratory was defined as ³H-5-HT binding in the presence of 100 nM 8-OH-DPAT plus 3,000 nM mianserin. 5-HT₁C binding in rat frontal cortex was defined as specific ³H-5-HT binding in the presence of 100 nM 8-OH-DPAT plus 10 nM RU 24969. Under these conditions, pharmacological competition studies reveal that each putative 5-HT₁ binding-site subtype has a distinct pharmacological profile (Table 1). The 5-HT₁B site has extremely high affinity for RU 24969 (0.38 nM) and relatively high affinity for TFMPP. The 5-HT₁C site has relatively high affinity for 5-HT, mianserin, and methysergide.

However, a species study revealed interesting variations in the presence of 5-HT₁ binding site subtypes (Heuring et al., 1986). In both the rat and mouse, RU 24969 distinguishes two distinct subpopulations of non-5-HT₁A binding sites. In contrast, RU 24969 interactions with non-5-HT₁A binding in guinea pig, dog, human, cow, chicken, turtle, and frog brain membranes are consistent with a single population non-5-HT₁A binding-site subtype. These results suggest that certain 5-HT₁ binding-site subtypes are species specific. In particular, the 5-HT₁B site (as defined above) appears to be unique to rat and mouse brain membranes.

These data are consistent with the findings of multiple other laboratories. A number of radioligands have been shown to label the 5-HT₁A binding site: ³H-8-OH-DPAT (Gozlan et al., 1983; Hall et al., 1985; Peroutka, 1985), ³H-TVX Q 7821 (Dompert et al., 1985), ³H-buspirone (Moon & Taylor, 1985), and ³H-1-(2-(4-aminophenyl)ethyl)-4-(3-trifluoromethylphenyl) piperazine (PAPP) (Asarch et al., 1985). In addition, ³H-WB 4101, previously considered a selective alpha₁-adrenergic radioligand, has been demonstrated to label the 5-HT₁A site (Norman

### Table 1. Characteristics of 5-HT₁A, 5-HT₁B, and 5-HT₁C Binding-Site Subtypes

<table>
<thead>
<tr>
<th>Radiolabeled by</th>
<th>5-HT₁A</th>
<th>5-HT₁B</th>
<th>5-HT₁C</th>
</tr>
</thead>
<tbody>
<tr>
<td>²H-5-HT</td>
<td>²H-5-HT</td>
<td>²H-5-HT</td>
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</tr>
<tr>
<td>²H-8-OH-DPAT</td>
<td>¹³CYP</td>
<td>²H-Mesulergine</td>
<td></td>
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<tr>
<td>²H-TVX Q 7821</td>
<td>RU 24969</td>
<td>¹⁵LSD</td>
<td></td>
</tr>
<tr>
<td>²H-WB 4101</td>
<td>²H-Buspirone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>²H-Buspirone</td>
<td>²H-PAPP</td>
<td></td>
<td></td>
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<tr>
<td>High-Density Regions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raphe nuclei</td>
<td>Dorsal Subiculum</td>
<td>Choroid Plexus</td>
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</tr>
<tr>
<td>Hippocampus</td>
<td>Globus Pallidus</td>
<td></td>
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<tr>
<td>Substantia Nigra</td>
<td>5-HT</td>
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<td></td>
</tr>
<tr>
<td>Potent Pharmacologic Agents</td>
<td>5-HT</td>
<td>5-HT</td>
<td>5-HT</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>RU 24969</td>
<td>Mesulergine</td>
<td></td>
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<tr>
<td>5-MeDMT</td>
<td></td>
<td>Mianserin</td>
<td></td>
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<tr>
<td>TVX Q 7821</td>
<td></td>
<td>Methysergide</td>
<td></td>
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<tr>
<td>Buspirone</td>
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et al., 1985). Regardless of the H-ligand used to label the site, it displays high and selective affinity for 8-OH-DPAT, 5-methoxydimethyltryptamine (5-MeDMT), TVX Q 7821, and buspirone. The 5-HT\textsubscript{IA} site is densely present in the CA1 region and dentate gyrus of the hippocampus and in the raphe nuclei (Glaser et al., 1985; Pazos & Palacios, 1985). In addition, the fact that 5,7-dihydroxytryptamine-induced lesions of the raphe system cause a loss of \textsuperscript{3}H-8-OH-DPAT binding in the striatum but not hippocampus has led Hamon and colleagues to hypothesize that \textsuperscript{3}H-8-OH-DPAT may also label a presynaptic 5-HT autoreceptor (Gozlan et al., 1983).

The putative 5-HT\textsubscript{IB} site has been labeled in rat brain with \textsuperscript{125}I cyanopindolol (Hoyer et al., 1985a; Pazos et al., 1985). The \textsuperscript{125}I cyanopindolol (\textsuperscript{125}ICYP) site has high affinity for 5-HT and RU 24969 and relatively low affinity for d-LSD and 8-OH-DPAT. The highest densities of 5-HT\textsubscript{IB} sites in rat brain are found in the caudate nucleus, superior colliculus, lateral geniculate body, subiculum, and substantia nigra (Pazos & Palacios, 1985a).

The 5-HT\textsubscript{IC} site was first characterized in membranes from pig choroid plexus and cortex (Pazos et al., 1984). The site was labeled by both \textsuperscript{3}H-5-HT and \textsuperscript{3}H-mesulergine. Independently, Yagaloff and Hartig (1985) labeled the site with \textsuperscript{125}I LSD in the rat choroid plexus. The site has high affinity for 5-HT, methysergide, and mianserin and relatively low affinity for RU 24969. Another 5-HT\textsubscript{IC} binding-site subtype having low affinity for RU 24969 has been identified in the bovine striatum (Peroutka, 1985). The relationship between putative 5-HT\textsubscript{IC} sites in rat, bovine, and pig brain is currently being investigated in this laboratory.

A number of functional correlates of the 5-HT\textsubscript{1} class of binding sites have been proposed (Peroutka, 1984) (Table 2). In particular, the extensive characterization of the 5-HT\textsubscript{IA} binding-site subtype has enabled an analysis of this putative receptor in many 5-HT-mediated effects. For example, the ability of 5-HT\textsubscript{IA} selective drugs to stimulate a 5-HT sensitive cyclase (Shenker et al., 1985) and the potent effects of guanosine triphosphate (GTP) on \textsuperscript{3}H-8-OH-DPAT binding (Schlegel & Peroutka, in press) suggest a linkage of the 5-HT\textsubscript{IA} site to an adenylate cyclase. A role for the 5-HT\textsubscript{IA} site has been proposed for the mediation of certain components of the 5-HT behavioral syndrome (Smith & Peroutka, in press; Tricklebank et al., in press), canine basilar artery contractions (Peroutka, Huang, et al., in press; Taylor et al., in press), the potentiation of ejaculation and/or seminal emissions (Kwong et al., in press), hypothermia (Gudelsky et al., 1985; Middlemiss et al., 1985), and hypotensive effects of 5-HT (Doods et al., 1985).

The interactions of 5-HT\textsubscript{IA} selective agents have also been analyzed in an in vitro rat hippocampal slice preparation. Application of 8-OH-DPAT, buspirone, or TVX Q 7821 leads to a dose-dependent decrease in the amplitude of the population spike (Peroutka, Mauk, et al., in press). Ketanserin, a selective 5-HT\textsubscript{IB} antagonist, has no independent effect on the amplitude of the population spike. In addition, ketanserin has no effect on the buspirone or TVX Q 7821-induced inhibition of the CA1 population spike. Therefore, modulation of hippocampal CA1 neuronal activity may be a functional correlate of 5-HT\textsubscript{IA} receptor activation.

The 5-HT\textsubscript{IB} site has recently been analyzed in terms of the 5-HT "autoreceptor." A significant correlation has been found between the potencies of drugs at the 5-HT\textsubscript{IB} site as labeled by \textsuperscript{125}I cyanopindolol and the "autoreceptor" (Engel et al., 1986). Finally,

### TABLE 2. Proposed Functional Correlates of 5-HT\textsubscript{1} Binding-Site Subtypes

<table>
<thead>
<tr>
<th>5-HT\textsubscript{IA}</th>
<th>5-HT\textsubscript{IB}</th>
<th>5-HT\textsubscript{IC}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylate cyclase stimulation</td>
<td>Forepaw treading, tremor, head-weaving</td>
<td>Phosphoinositide turnover</td>
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<tr>
<td>Canine basilar artery contractions</td>
<td>Facilitation of ejaculation and/or seminal emissions</td>
<td>&quot;Autoreceptor&quot;</td>
</tr>
<tr>
<td>Hypotensive effects</td>
<td>Hypothermia</td>
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<tr>
<td>CA1 Hippocampal inhibition</td>
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preliminary studies suggest that phosphoinositide turnover may be mediated by the 5-HT_1C sites in rat choroid plexus (Sanders-Bush & Conn, 1986).

Clearly, significant progress in the analysis of 5-HT binding-site subtypes has been made in the past few years. Future studies will be directed at obtaining more selective radioligands or relatively "pure" regions or species. Ideally, a radioligand should label a homogeneous population of receptors. Finally, although substantial progress is being made, the role of 5-HT in the CNS is still quite unclear. Functional correlates of 5-HT binding-site subtypes may significantly add to the understanding of the role of 5-HT in the CNS.

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References


